

ISO

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION

ISO RECOMMENDATION R 937

MEAT AND MEAT PRODUCTS

DETERMINATION OF NITROGEN CONTENT

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BRIEF HISTORY

The ISO Recommendation R 937, *Meat and meat products – Determination of nitrogen content*, was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, the Secretariat of which is held by the Magyar Szabványügyi Hivatal (MSZH).

Work on this question by the Technical Committee led, in 1966, to the adoption of a Draft ISO Recommendation.

In April 1967, this Draft ISO Recommendation (No. 1233) was circulated to all the ISO Member Bodies for enquiry. It was approved, subject to a few modifications of an editorial nature, by the following Member Bodies :

Australia	India	Romania
Bulgaria	Iran	South Africa, Rep. of
Colombia	Ireland	Thailand
Czechoslovakia	Israel	Turkey
France	Korea, Rep. of	U.A.R.
Germany	Norway	United Kingdom
Greece	Poland	U.S.S.R.
Hungary	Portugal	Yugoslavia

Two Member Bodies opposed the approval of the Draft :

Netherlands
New Zealand

The Draft ISO Recommendation was then submitted by correspondence to the ISO Council, which decided, in January 1969, to accept it as an ISO RECOMMENDATION.

MEAT AND MEAT PRODUCTS

DETERMINATION OF NITROGEN CONTENT

1. SCOPE

This ISO Recommendation describes a reference method for the determination of the nitrogen content of meat and meat products.*

2. DEFINITION

By *nitrogen content*, is meant the quantity of nitrogen corresponding to the ammonia produced and determined under the conditions described.

3. PRINCIPLE

Digestion of the product with concentrated sulphuric acid, using copper (II) sulphate as a catalyst, to convert organic nitrogen to ammonium ions, addition of alkali, distillation of the liberated ammonia into an excess of boric acid solution; titration with hydrochloric acid to determine the ammonia bound by the boric acid, and calculation of the nitrogen content of the sample from the amount of ammonia produced.

4. REAGENTS

All reagents should be of analytical quality.

Water should be distilled water or water of at least equal purity.

- 4.1 *Copper (II) sulphate*, ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).
- 4.2 *Potassium sulphate*, (K_2SO_4), anhydrous.
- 4.3 *Sulphuric acid*, $\rho_{20} = 1.84$ g/ml.
- 4.4 *Sodium hydroxide solution*, carbonate-free, containing approximately 33 g of sodium hydroxide (NaOH) per 100 g of solution. Prepare by dissolving 500 g of sodium hydroxide (NaOH) in 1000 ml of water.
- 4.5 *Boric acid solution*. Dissolve 40 g of boric acid (H_3BO_3) in water and dilute to 1000 ml.
- 4.6 *Hydrochloric acid*, 0.1 N standard volumetric solution, the normality being recorded to four places of decimals.

* See also ISO Recommendation R . . . , *Agricultural food products – General directives for the determination of nitrogen by the Kjeldahl method* (at present at the stage of a draft proposal).

- 4.7 *Indicator solution*, mixed indicator (methyl red–methylene blue)*, prepared by dissolving 2 g of methyl red and 1 g of methylene blue in 1000 ml of ethanol 95 % (v/v).

The colour change of this indicator solution occurs at a pH of 5.4. Store the indicator solution in a brown bottle in a dark and cool place.

4.8 *Boiling regulators*

4.8.1 *For the digestion*. Glass beads, silicon carbide or splinters of hard porcelain.

4.8.2 *For the distillation*. Silicon carbide or freshly ignited pieces of pumice stone.

5. APPARATUS

Usual laboratory apparatus not otherwise specified, and the following items :

- 5.1 *Mechanical meat mincer*, laboratory size, fitted with a plate with holes of diameter not exceeding 4 mm.
- 5.2 *Greaseproof paper*, pieces about 9 cm X 6 cm.
- 5.3 *Burette*, 50 ml, class A according to ISO Recommendation R 385, *Burettes*.
- 5.4 *Kjeldahl flask*, of not more than 800 ml capacity, provided, if desired, with a pear-shaped glass bulb loosely fitting on top of the neck.
- 5.5 *Steam distillation apparatus* or, alternatively, ordinary distillation apparatus.
- 5.6 *Heating device*, on which the Kjeldahl flask can be heated in an inclined position, in such a way that the source of heat only touches that part of the flask wall which is below the liquid level. For gas heating a suitable device is a plate of asbestos provided with a circular hole, so that only the lower part of the flask is exposed to the free flame.
- 5.7 *Effective suction device*, for the acid fumes evolved during the digestion.
- 5.8 *Analytical balance*.

6. SAMPLE

- 6.1 Proceed from a representative sample of at least 200 g, (see ISO Recommendation R . . .**, *Meat and meat products – Sampling*).
- 6.2 Store the sample in such a way that deterioration and change in composition are prevented. Preservatives, if any, should not contain nitrogen compounds in measurable amounts.

* Sometimes known as *Tashiro* indicator.

** In preparation.

7. PROCEDURE

7.1 Preparation of sample

Make the sample homogeneous by passing it at least twice through the meat mincer (5.1) and mixing. Keep it in a completely filled air-tight closed container and store it in such a way that deterioration and change in composition are prevented. Analyse the sample as soon as possible, but in any case within 24 hours.

7.2 Test portion

Place a few boiling regulators (4.8) into the Kjeldahl flask (5.4), then add about 15 g of anhydrous potassium sulphate (4.2) and 0.5 g of copper (II) sulphate (4.1).

Weigh, to the nearest 0.001 g, about 2 g of the prepared sample (or 1.5 g in the case of a sample rich in fat) on a piece of parchment (5.2).

Transfer the parchment and the test portion to the Kjeldahl flask.

7.3 Determination

Add 25 ml of sulphuric acid (4.3) to the Kjeldahl flask. Mix by gently swirling the liquid. If desired, a pear-shaped glass bulb may be inserted into the neck of the flask with its tapering end downwards.

Place the flask in an inclined position (at an angle of about 40° from the vertical position) on the heating device (5.6). At first heat the flask gently until the foaming has ceased and the contents have become completely liquefied. Then digest by boiling vigorously, occasionally rotating the flask, until the liquid has become completely clear and of a light blue-green colour. Keep the liquid boiling for another 1 hour 30 minutes.

The total digestion time should not be less than 2 hours. Take care that no condensed liquid runs down the exterior of the flask. Prevent the escape of too much sulphuric acid caused by overheating during the digestion, as this results in a loss of nitrogen.

Cool to about 40 °C and add cautiously about 50 ml of water. Mix and allow to cool.

Pour into a conical flask, of capacity about 500 ml, 50 ml of the boric acid solution (4.5) from a measuring cylinder, add four drops of the indicator solution (4.7), mix and place the flask under the condenser of the distillation apparatus (5.5) so that the outlet of the adapter dips into the liquid.

Treat the contents of the Kjeldahl flask in one of the following ways :

- (a) *In the case of steam distillation.* Transfer the contents of the Kjeldahl flask to the distillation apparatus and rinse the flask with about 50 ml of water. Add 100 ml of the sodium hydroxide solution (4.4) by means of a measuring cylinder. Pour carefully along the inclined neck of the flask so that the two layers in the flask do not mix. Immediately attach the flask to the splash head of the distillation apparatus. Heat the alkaline liquid by passing steam through it until boiling and keep it so for 20 minutes. At first heat gently to reduce foaming. The collected volume of distillate should be at least 150 ml.

- (b) *In the case of ordinary distillation.* Cautiously dilute the contents of the Kjeldahl flask with about 300 ml of water and swirl. If desired, transfer to a one-litre flask. After about 15 minutes add 100 ml of the sodium hydroxide solution (4.4) by means of a measuring cylinder, carefully along the inclined neck of the flask so that the two layers in the flask do not mix. Immediately attach the flask to the splash-head of the distillation apparatus.

Distil at least 150 ml of liquid, even if the mixture bumps irregularly. Continue the distillation until the mixture starts bumping or until 250 ml of distillate has been collected. Make sure that the distillate is cooled effectively and prevent the boric acid solution from becoming warm.

In either case, lower the conical flask just before terminating the distillation, so that the outlet of the adapter is above the liquid level. Rinse the outlet of the adapter above the liquid (internally and externally) with a little water. Verify the completion of the ammonia distillation with a red litmus paper, wetted with distilled water; its colour should not be affected by the liquid dripping from the condenser. Stop heating. If the distillation is found to be incomplete, carry out a new determination, carefully following the instructions.

Titrate the contents of the conical flask with the hydrochloric acid solution (4.6). Record the number of millilitres of hydrochloric acid solution required, estimated to the nearest 0.02 ml.

Carry out two determinations on the same prepared sample.

7.4 Blank test

Always perform a blank test (in duplicate) when fresh batches of reagents or freshly prepared solutions are used. It is advisable to carry out a blank test occasionally for reagents and solutions which have already been in use for some time.

Carry out this blank test according to clause 7.3, taking a piece of parchment (5.2) only.

7.5 Remark

It is also possible to determine the nitrogen in an aliquot part of the contents of the Kjeldahl flask. Under these conditions, suitable modifications to the apparatus and procedure will be required (quantities and concentrations of the reagents used, time of distillation, volume of distillate).

These modifications should be shown in the test report.

8. EXPRESSION OF RESULTS

8.1 Method of calculation and formula

The nitrogen content, as a percentage by mass of the sample, is equal to :

$$0.0014 \times (V_1 - V_0) \times \frac{100}{M}$$

where

V_0 is the volume, in millilitres, of 0.1 N hydrochloric acid solution required for the blank test,

V_1 is the volume, in millilitres, of 0.1 N hydrochloric acid solution required for the test portion,

M is the mass, in grammes, of the test portion.

NOTE. — If the standard volumetric solution of hydrochloric acid used is not of exactly the strength indicated in clause 4.6, a suitable correction factor should be used in calculating the result.

Take as the result the arithmetic mean of the two determinations, if the requirements of repeatability are satisfied. Report the nitrogen content as a percentage, by mass, to the nearest 0.01 g of nitrogen per 100 g of sample.

8.2 Repeatability

The difference between the results of duplicate determinations performed almost simultaneously or in rapid succession by the same analyst should not be more than 0.10 g of nitrogen per 100 g of sample.

9. NOTES ON PROCEDURE

9.1 The determination should be carried out in a room free from ammonia vapour.

9.2 Nitrogen originating from non-protein compounds will be included in the determination and will give inaccurate results for the protein content if the protein is calculated from the nitrogen content.

If, in addition to the result in terms of nitrogen, it is desired to express the results as protein, it is necessary to indicate the factor used.

10. TEST REPORT

The test report should show the method used and the result obtained, as nitrogen. If the result in terms of protein is also required, the factor used should also be given. It should mention any operating conditions not specified in this ISO Recommendation, or regarded as optional (in particular, if the determination has been carried out on an aliquot portion, see clause 7.5), as well as any circumstances that may have influenced the result.

The report should include all details required for complete identification of the sample.
